REMARKS

The Applicants appreciate the Examiner's thorough examination of the subject application. Applicants request reconsideration of the subject application based on the following remarks.

After entry of the instant amendment, claims 8, 10-12, 18, and 20-22 are pending. Claims 1-7, 9, 13-17, and 19 have been cancelled, and claims 8, 12, 18, and 22 have been amended. Support for the amendments to claims 8, 12, 18, and 22 can be found throughout the specification. No new matter has been added by virtue of these amendments. Applicants reserve the right to pursue the subject matter cancelled from the claims in this or a subsequent application.

Claims 8, 13, and 18 were objected to allegedly because of various informalities set forth on page 2 of the November 1, 2004 office action.

The amendments to claims 8 and 18 and the cancellation of claim 13, obviate the objections to the claims.

Claims 8, 10-13, 15-18, and 20-22 were rejected under 35 U.S.C. §112, second paragraph, as being allegedly indefinite for failing to particular point out and distinctly claim the subject matter which applicant regards as the invention.

The Office Action has maintained the previously presented rejection due to the lack of clarity in the step of comparing the mutant and wild-type. The claims, as amended, particularly point out and distinctly claim methods comprising a step of "comparing a phenotype of the mutant individuals of the vertebrate animal with that of wild type individuals of the vertebrate animal..."

The office action has also rejected claims 8 and 18 based on several new §112, second paragraph, grounds.

Claims 8, as amended, provides in step (d), growing the fertilized eggs to mutant embryos of the vertebrate animal, wherein the mutant embryos comprise a gene having a small deletion of a plurality base pairs at the crosslinked site in a genome. More particularly, step (d) provides that the mutant embryos have a *small* deletion in the genome, which deletion comprises at least two base pairs (e.g., a *plurality*), and the deletion is at the point of crosslinking between the psoralen derivative and the DNA double helix. Thus, the claim language, as presently amended particularly points out and distinctly claims the subject matter of the claimed invention.

Claims 8 and 18, as amended, comply with all the requirements of 35 U.S.C. §112, including the requirements of §112, second paragraph. Claims 10-12 and 20-22 depend from claims 8 and 18, and thus also satisfy the requirements of 35 U.S.C. §112, including the requirements of §112, second paragraph.

Withdrawal of the §112, second paragraph, rejections is thus requested.

Claims 8, 10-13, 15-18, and 20-22 were rejected under 35 U.S.C §103 (a) as being allegedly anticipated by Chakrabarti (*Genetics*, 1983, 103:109-123), Grunwald #1 (*Genet. Res.*, 1991, 59:93-101), Grunwald #2 (*Genet. Res.*, 1001, 59:103-116) taken with Thomas (*Mol. Cell. Biol.*, 1996 16(5):2537-2544).

The rejection is traversed.

Claims 8 and 18, as amended, provide methods of preparing a mutant embryo of a vertebrate animal and/or analyzing a function of a gene of a vertebrate animal in which a gene is mutated by effecting a small, multiple base-pair deletion.

Applicants have surprisingly discovered that psoralen derivatives causes a mutation in a vertebrate sperm cell's genome upon exposure to UV-light. More particularly, Applicants have discovered that irradiating a mixture of DNA and a psoralen derivative with UV light induces a small deletion of a plurality of base-pairs in the vertebrate's genome.

None of the cited references alone or in combination teach or suggest the methods of the invention presented in the instant amendment.

As the reference is understood, Chakrabarti et al. recites that "several observation reported here suggested that an appreciable fraction of γ–ray-induced zebrafish mutations are **long deficiencies**" (See page 120 line 6-8 in Chakrabarti et al., emphasis added). Thus, the long genome deficiencies induced by exposure to γ–ray irradiation taught by Chakrabarti make it difficult to isolate a mutation at a level of single gene locus.

Grunwald et al. #1 teaches mutation methods using UV light which induce **point mutations**, frameshift mutations, but rarely large deletions or mutations (See page 93, right column, line13-15).

Grunwald et al. #2 recites that "ENU-induced mutations are probably point mutations" (See page 115, paragraph(iv)). Applicants note that cloning of point mutated genes typically requires laborious procedures such as genetic mapping and chromosomal walking.

Thomas, et al, fails to overcome the limitations of the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2.

As the reference is understood, Thomas recites the mechanism of mutagenicity of psoralen by using a human cell extract. In brief, He examines the efficiency and fidelity of *simian virus 50 origin–dependent replication in a human cell extract of M13 mp2 DNA* treated with psoralen plus irradiation. Such SV40 origin-dependent replication is never the same as the replication occurring in fertilized eggs of a vertebrate which are generated from eggs and sperms treated with psoralen and the mutation is supposed to be fixed through the segmentation of the

fertilized eggs. Thus, one of ordinary skill in the art would expect that a small deletion as demonstrated by Thomas will occur in the fertilized vertebrate eggs.

The office action relies upon the combination of each of Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 in combination of Thomas to obtain the instant invention. More particularly, the office action appears to aver that one of ordinary skill in the art would be motivated to combine the use of psoralen/UV irradiation taught by Thomas to mutate DNA in a cellular extract with the methods of mutating sperm with high energy γ-ray, or UV irradiation or chemically induced mutation recited by Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2.

Thus, the office action appears to take the position that one of ordinary skill in the art would be motivated and have a reasonable expectation of success to modify the mutation methods recited by Chakrabarti, Grunwald #1, or Grunwald #2 by replacing the mutagenesis agent recited therein with any other mutagenesis agent used to mutate any DNA sequence.

Applicants respectfully point out that Thomas teaches methods of mutating human DNA which is present in a **cellular extract** by contacting the extract with a psoralen derivative and UV irradiation. The Office Action has provided any motivation or basis for the interchangeability of mutagenesis agents suitable for *in vivo* and *in vitro* mutation methods. Thus, one of ordinary skill in the art would not be motivated from the Thomas disclosure to substitute the disclosed psoralen/UV irradiation mutagenesis agent into the methods of mutating, *in vivo*, sperm cells recited by Chakrabarti, Grunwald #1, or Grunwald #2.

One of ordinary skill in the art would not have been motivated to combine the methods of mutating sperm cells recited by Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 with the methods of mutating DNA present in a cellular extract recited by Thomas. More particularly, it would be difficult for one of skill in the art to predict if a small deletion would occur in fertilized eggs of vertebrates through the segmentation if the mutagenesis agent of Thomas was used in the methods of Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2. Moreover,

even if one were to allege that such a combination is supported by the disclosure of the cited documents, one of ordinary skill would not have a reasonable expectation of success without impermissible hindsight.

Accordingly, the present invention would not be obvious to anyone skilled in the art from any of Chalrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 taken with Thomas et al. Applicants request withdrawal of the rejection and reconsideration of the claims.

Claims 8, 10-13, 15-18, and 20-22 were rejected under 35 U.S.C §103 (a) as being allegedly anticipated by Chakrabarti (*Genetics*, 1983, 103:109-123), Grunwald #1 (*Genet. Res.*, 1991, 59:93-101), Grunwald #2 (*Genet. Res.*, 1001, 59:103-116) taken with Yandell (*PNAS*, 1994, 91, pages 1381-1385).

The rejection is traversed.

Yandell, et al, fails to overcome the limitations of the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2 as discussed *supra*.

As the reference is understood, Yandell studies the mutagenic spectrum of psoralen in nematode Caenorhabditis elegance (C. elegance) which has homozygote and heterozygote and thus is **not** a vertebrate. Further, in Yandell, C. elegance individuals not sperm cells of the species are treated with psoralen. Thus, one of ordinary skill in the art would not have a reasonable expectation that the process for fixing the mutation induced by psoralen in C. elegance individuals is the same or different from the process in fertilized vertebrate eggs wherein the mutation is supposed to be fixed through the segmentation of the fertilized eggs.

Thus, the office action appears to take the position that one of ordinary skill in the art would be motivated and have a reasonable expectation of success to modify the mutation methods recited by Chakrabarti, Grunwald #1, or Grunwald #2 by replacing the mutagenesis agent recited therein with any other mutagenesis agent used to mutate any DNA sequence.

Applicants respectfully point out that Yandell teaches methods of mutating human DNA which is present in a **intact organism** by contacting the intact nematode with a psoralen derivative and UV irradiation. The Office Action has provided any motivation or basis for the interchangeability of mutagenesis agents suitable for *in vivo* and *in vitro* mutation methods. Thus, one of ordinary skill in the art would not be motivated from the Yandell disclosure to substitute the disclosed psoralen/UV irradiation mutagenesis agent into the methods of mutating, *in vivo*, sperm cells recited by Chakrabarti, Grunwald #1, or Grunwald #2.

One of ordinary skill in the art would not have been motivated to combine the methods of mutating sperm cells recited by Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 with the methods of mutating DNA present in a cellular extract recited by Yandell. More particularly, it would be difficult for one of skill in the art to predict if a small deletion would occur in fertilized eggs of vertebrates through the segmentation if the mutagenesis agent of Yandell was used in the methods of Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. #2. Moreover, even if one were to allege that such a combination is supported by the disclosure of the cited documents, one of ordinary skill would not have a reasonable expectation of success without impermissible hindsight.

Accordingly, the present invention would not be obvious to anyone skilled in the art from any of Chalrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 taken with Yandell et al. Applicants request withdrawal of the rejection and reconsideration of the claims.

Reconsideration and withdrawal of the rejection of the noted claims are thus requested.

It is believed the application is in condition for immediate allowance, which action is earnestly solicited.

Respectfully submitted,

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